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Differential activation of memory-relevant brain regions during a dialysis cycle

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Cognitive impairment is a common and largely undiagnosed finding in a significant number of dialysis patients. These alterations may result from concomitant cerebrovascular disease, hemodynamic instability, the uremic milieu, or changes induced by the dialysis process. In order to gain further insight into this, we recruited 12 stable chronic hemodialysis patients (without clinical neurological disease) and an age- and gender-matched cohort of 12 control individuals (without renal or neurological problems) in a prospective, single-center study. In order to disentangle the influence of dialysis itself on memory function, each dialysis patient was tested twice: once immediately before dialysis following a long weekend (t1) and again the day after this dialysis (t2). The control individuals were tested in the same time frame. Neuropsychological testing found that the control individuals performed significantly better in verbal learning, motor speed, task switching, verbal comprehension, word fluency, spatial visualization, spatial perception, and reasoning; all independent of the time point. Functional magnetic resonance imaging of the whole brain in seven hemodialysis patients found significantly more bilateral activation of the hippocampus during the verbal working memory task at t2 relative to t1 compared with their seven matched control counterparts. Thus, our study found differential and task-specific activation of memory-relevant brain areas during a dialysis cycle.

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To date only a limited number of studies have investigated cognitive functions in dialysis patients.[1,2] Recent reports in US hemodialysis (HD) patients suggest that cognitive impairment is a common and largely undiagnosed finding.[3] However, the pathogenesis of cognitive impairment in dialysis patients remains obscure. Concomitant diseases such as microangiopathy are likely to contribute to the cognitive impairments observed: structural brain analyses performed in patients with chronic renal insufficiency as well as in dialysis patients using magnetic resonance imaging (MRI) techniques[1] suggest that even in dialysis patients who show no clinical evidence of any neurological disease, MRI detects significantly more white matter hyperintensities,[1,4] cerebral microbleeds,[5] and subclinical infarcts.[6,7]

Yet, the fact that cognitive functions may vary significantly over the course of a dialysis cycle, with most cognitive processes being worst during dialysis,[8] strongly suggests that direct consequences of chronic renal failure such as overhydration and/or the uremic milieu may also effect on cognitive processing.[1] Functional MRI (fMRI) may be used to further examine these issues.

fMRI is a technique for measuring brain activity by detecting changes in blood oxygenation that occur in response to neural activity. This technique has dramatically improved our understanding of the neural mechanisms underlying cognitive functions such as, memory,[9,10] attention,[11,12] or executive functions.[13] On the basis of the previous data indicating a particular impairment of verbal memory in dialysis patients,[8] we combined an fMRI verbal working memory task with structural MRI, blood chemistry, and a detailed neuropsychological assessment at two different time points during the course of dialysis. We recruited a cohort of stable chronic HD patients without clinical neurological disease and an age- and sex-matched control (Con) cohort without renal or neurological disease. To disentangle the influence of dialysis itself on memory function, each dialysis patient/participant was analyzed twice: once immediately before dialysis (after a long weekend dialysis

interval) and once the day after dialysis. This is the first fMRI study in dialysis patients indicating that memory-relevant brain areas are differentially activated in dialysis patients dependent on the course of the dialysis cycle.

RESULTS

Descriptive and clinical data

HD patients and healthy Cons were matched for sex, age, body weight, education, and handedness (Table 1). Significant differences between these two groups were changes in body weight, hematocrit, and hemoglobin between the two time points analyzed (Table 1). These differences reflect the significant fluid removal of approximately 3 l during the dialysis session.

All HD patients and Cons were able to participate in the different tests used for the neuropsychological assessment. However, at t1 some HD patients had to stop their assessment before all tests could be finished, since they had to be transported to their scheduled dialysis appointments. Thus, the number of patients included in the respective neuropsychological tests is specified in Table 2. The fMRI experiments were performed in 12 dialysis patients and 12 healthy Cons. However, fMRI data of five dialysis patients had to be excluded from further analysis. Three patients showed movement artefacts >4 mm in at least one of the two measurements, and two patients were excluded because of technical problems (in one patient no log file was recorded and one further patient used the wrong buttons of the answering panel). Parallel to the exclusion of HD patients, we also excluded the respective sex- and age-matched Con subjects from further fMRI analysis. Thus, for the fMRI part of this study, data of seven male HD (mean age 43.6 ± 6.6 years) and seven age-matched male Con (mean age 43.4 ± 6.7 years) subjects were analyzed.

Table 1 | Clinical and demographic data of hemodialysis patients and controls

	Hemodialysis	Controls	Significance
n	12	12	
Sex (male:female)	11:1	11:1	
Age (years)			
Min–max	20–59	22–58	
Mean ± s.d.	45.0 ± 11.5	44.7 ± 10.7	n.s.
Years of education			
Mean ± s.d.	13.1 ± 2.5	15.1 ± 1.8	n.s.
Handedness (laterality quotient)			
Mean ± s.d.	81.3 ± 15.6	85.4 ± 20.1	n.s.
Body weight, (t2, kg)			
Min–max	51.2–100.8	65.9–108	
Mean ± s.d.	76.7 ± 15.1	86.6 ± 13.9	n.s.[a]
Change in body weight (mean ± s.d., t2–t1, kg)	−2.9 ± 0.9	−0.3 ± 0.7	$P<0.05$
Hematocrit (mean ± s.d., t2, %)	42.6 ± 4.9	44.9 ± 3.1	n.s.[a]
Change in hematocrit (mean ± s.d., t2–t1, %)	3.5 ± 1.8	0.4 ± 1.2	$P<0.05$
Hemoglobin (mean ± s.d., t2, g/dl)	14.0 ± 1.8	15.4 ± 1.2	$P<0.05$[a]
Change in hemoglobin (mean ± s.d., t2–t1, g/dl)	1.1 ± 0.6[a]	0.0 ± 0.5	$P<0.05$

Abbreviation: n.s., no significant difference.
[a]*Post hoc T*-tests.

fMRI study – reaction times and error rates

We analyzed the reaction times and error rates during the verbal working memory task at the two time points (t1, t2) and for the two groups (HD, Con). Dependent variables were the mean of median reaction times and the mean of median error rates averaged over all trials for each condition. HD and Con reacted significantly faster at t2 indicating a practice effect of this task ($P<0.01$; Figure 1). No significant differences were identified when comparing the HD and Con groups independent of the time points or when analyzing the interaction between time point and group. Analyzing the error rates of HD and Con at the two time points did not reveal any significant differences, neither as effect of time or group, nor the interaction thereof (Figure 1). In addition, we analyzed the error rates during the Con task (i.e., letter reading task). There was no significant difference between HD and Con or between time points. Similarly, no significant interaction was observed. The percentages of correct answers were $98.8 \pm 1.9\%$ (HD, t1), $99.4 \pm 1.5\%$ (Con, t1), $98.8 \pm 1.9\%$ (HD, t2), and 100% (Con, t2). Reaction times were not calculated for the control letter reading task, because subjects were not asked to press the button as fast as possible.

fMRI study – neural activations

We analyzed changes in neural activity of the whole brain during the verbal working memory and the control letter reading task at the two time points (t1, t2) and for the two groups (HD, Con). Comparing the activation signals of the two groups independent of the time revealed no significant differences for both tasks. Similarly, comparing the activation signals of the two time points independent of group revealed no significant differences for both tasks. In contrast, however, the interaction of group and time showed significant activations in the hippocampal regions bilaterally (Figure 2) for the verbal working memory task only. The difference between activations of Con at t1 and HD at t1 was significantly different from the difference of activation of Con at t2 and HD at t2 (Con t1 > HD t1) > (Con t2 > HD t2) in two areas located in the right and the left hippocampus (X, Y, and Z coordinates of the Montreal Neurological Institute (MNI) space: right: $X=18$, $Y=-14$, $Z=-15$; activated cluster size in voxel: 163, $P<0.01$, and left: $X=-22$, $Y=-8$, $Z=-17$; activated cluster size in voxel: 111, $P<0.05$; Figure 2). The plots in Figure 3 show the relationship of the parameter estimates for the four conditions (Con t1, HD t1, HD t2, and Con t2) suggesting an increased activation of the hippocampal region bilaterally after dialysis only in the HD patient group and only in the

Table 2 | Results of neuropsychological testing in 12 HD patients and 12 Cons

	t1		t2		
	HD, mean (s.d.)	Con, mean (s.d.)	HD, mean (s.d.)	Con, mean (s.d.)	ANOVA
Working memory					
Verbal (raw values)	6.0 (0.9)	6.2 (1.1)	6.1 (0.5)	6.3 (1.1)	
Spatial (raw values)	5.9 (0.8)	6.0 (1.0)	5.8 (0.7)	6.0 (0.9)	
Verbal memory					
Learning	50.1 (10.3)	57.9 (4.2)	49.3 (7.9)	56.0 (5.7)	g*
Delayed recall	49.0 (7.0)	50.5 (9.3)	47.0 (9.5)§	42.2 (9.8)§	
Recognition	47.0 (7.2)	51.1 (2.9)	41.2 (12.1)§; *n*=11	47.2 (8.3)§	t**
Figural memory	46.6 (29.7)	55.0 (30.7)	37.5 (26.7)	69.4 (29.7)	i*
Attention					
Visuomotor speed (s)	34.7 (13.2)	25.0 (4.5)	27.7 (7.8)	20.0 (4.09)	t**, g**
Task switching (s)	85.8 (25.9)	62.0 (20.2)	78.3 (29.3)	50.6 (17.7)	g**
Interference	55.2 (9.5); *n*=11	59.0 (6.2)	57.4 (8.0); *n*=10	63.3 (6.5)	t**
Speech					
Verbal comprehension	44.6 (9.9); *n*=11	54.2 (5.8)	46.4 (8.2); *n*=11	57.0 (4.9)	t*, g**
Word fluency	53.0 (8.4); *n*=9	63.6 (6.1)	55.0 (8.0); *n*=11	64.8 (7.3)	t*, g**
Perception					
Spatial visualization	42.2 (8.9); *n*=9	56.0 (5.4)	47.8 (7.4)	61.8 (4.4)	t**, g**
Perceptual speed	52.5 (2.7); *n*=6	61.4 (4.3)	52.7 (5.9)	64.5 (6.4)	t**, g**
Reasoning	47.0 (10.6) *n*=10	60.3 (6.6)	52.1 (9.9)	66.0 (6.5)	t**, g**
Dementia (screening/raw values)	29.1 (1.0)	29.5 (0.9)			
Depression (raw values)	13.3 (6.2); *n*=11	4.5 (4.4)			g**

Abbreviations: ANOVA, analysis of variance; Con, healthy control; HD, hemodialysis patient.
*$P<0.05$; **$P<0.01$. Reported are *t*-values and s.d. with a mean of 50 and an s.d. of 10 unless indicated otherwise.
Group sizes are always *n*=12 unless indicated otherwise.
i, significant interaction of groups and time; t, significant main effect of time; g, significant main effect of group; §, decline in delayed recall and recognition at t2 might be an interference effect of retesting using the identical interference word list in the parallel test version.

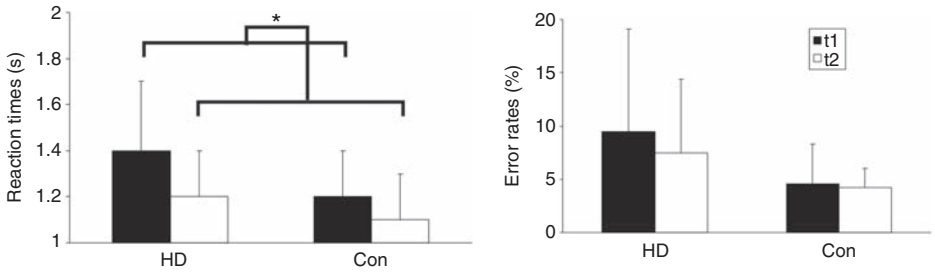

Figure 1 | Behavioral results of the verbal working memory functional magnetic resonance imaging (fMRI) task. Reaction times in sec (mean value ± s.d.) and error rates in % (mean value ± s.d.) for $n=7$ hemodialysis patients (HD) and $n=7$ age- and sex-matched control subjects (Con). Subjects showed a significant improvement in the reaction times at the time point t2 (t2, white boxes) compared with the time point t1 (t1, black boxes) independent of their group status, indicating a practice effect.

verbal working memory task but not in the control letter reading task. Whole-brain fMRI analysis of the control letter reading task detected no brain area significantly activated in any group or at any time.

Neuropsychological assessment

Significant group differences between HD and Con, independent of the analyzed time point, were detected for verbal learning, motor speed, task switching, verbal comprehension, word fluency, spatial visualization, spatial perception, and reasoning (Table 2). In all tasks wherein significant group differences were observed, Cons performed better than HD patients. The results of the verbal learning test are illustrated in Figure 3 as one representative example for the significant group differences between HD and Con. Significant time differences between t1 and t2, independent of the group affiliation were detected for verbal recognition, visuomotor speed, interference, verbal comprehension, word fluency, spatial visualization, perceptual speed, and reasoning (Table 2). All tasks that revealed significant time differences except for verbal recognition were performed better at t2, indicating practice effects underlying these differences.

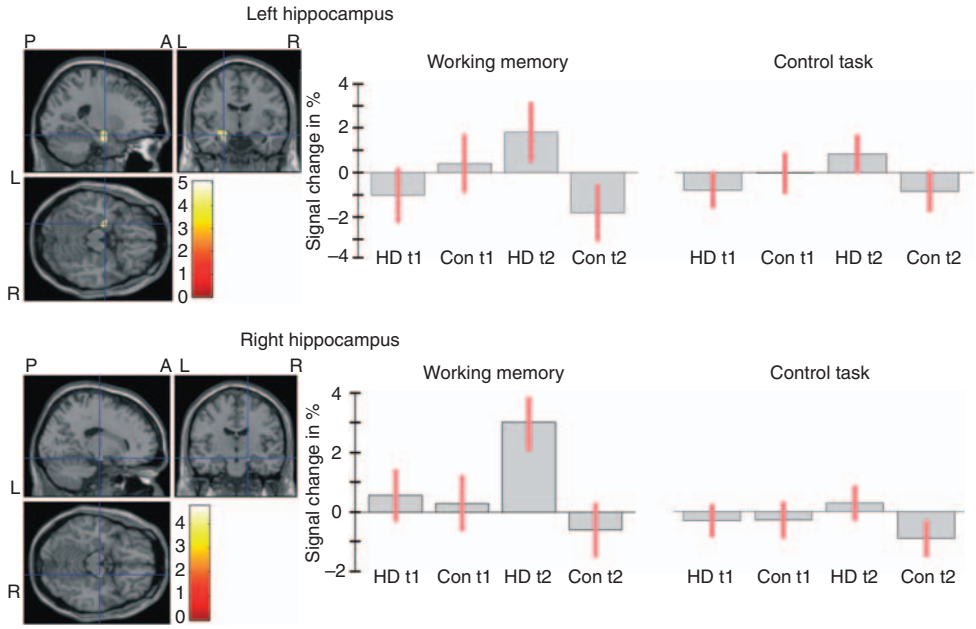

Figure 2 | Neural activation during the verbal working memory and the control functional magnetic resonance imaging (fMRI) task. Activations of the verbal working memory task are overlayed on a single-subject standard brain in MNI-space. The mean and standard error of mean percentage signal changes of the maximally activated voxels within the hippocampal activations during the verbal memory task (left (*XYZ*-coordinates: –22, –8, −17) and right (*XYZ*-coordinates: 18, –14, –15)) are plotted for the verbal working memory task and the control task for hemodialysis patients (HD, $n = 7$) and age- and sex matched control subjects (Con, $n = 7$) at time point 1 (t1) and time point 2 (t2). Significant activations of the analyzed hippocampal regions were only detected during the verbal working memory task. A, anterior; L, left; P, posterior; R, right.

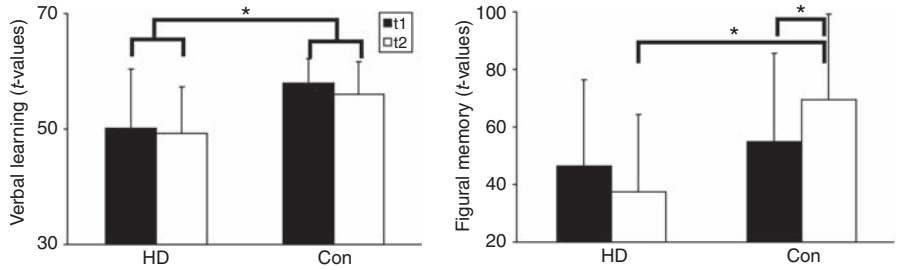

Figure 3 | Neuropsychological testing. *T*-values for verbal learning and figural memory for hemodialysis patients (HD, $n = 12$) and age- and sex-matched control subjects (Con, $n = 12$). In the verbal learning task Cons performed significantly better than HD patients independent of the time point. The figural memory test revealed a significant interaction between group and time. Cons improved their test results at time point t2 (t2, white boxes) while HD patients failed to show this practice effect.

The figural memory test was the only neuropsychological test that showed a significant interaction between time and group ($F(1,22) = 7.3$, $P = 0.01$) (Table 2 and Figure 3), that is, significant differences between patients and Cons at the two time points. Healthy Cons significantly improved their test results at t2 ($T\ (11) = 2.0$, $P = 0.03$), indicating a practice effect for this group while HD patients failed to improve their test results over time (Figure 3). Cons scored significantly higher at t2 in the figural memory test compared with the group of HD patients ($T\ (22) = -2.7$, $P = 0.01$).

HD patients scored significantly higher on the depression inventory than Cons (Table 2) $Z = 3{,}2$, $P < 0.01$. For the personality questionnaire, significant group differences were found in 2 of the 12 measurements. HD patients were significantly less satisfied with their personal living conditions (HD: 3.7 ± 2.3 stanine value, Con: 5.8 ± 1.4 stanine value; $T\ (19) = -23$; $P = 0.04$) and scored significantly higher on somatic disorders (HD: 5.8 ± 1.2 stanine value, Con: 4.3 ± 1.4 stanine value; $T\ (19) = 28$; $P = 0.01$).

DISCUSSION

Our results have a significant effect on our understanding of cognitive functions of dialysis patients. First, we were able to confirm and extend recent data from different cohorts of dialysis patients indicating that cognitive impairment is a common finding in dialysis patients affecting several different aspects of cognition. Second, and more importantly, we were able to extend these previous findings by showing for the first time that neural mechanisms associated with a cognitive task differ between HD patients and healthy Cons. Task-specific

differences in neural activity in right and left hippocampal areas were observed only in dialysis patients dependent on the time course of the dialysis cycle. Thus, our study introduces the use of fMRI as a powerful tool for the identification of the neural mechanisms underlying cognitive dysfunction in dialysis patients.

The verbal working memory fMRI experiment identified a significant interaction between time and group while the control letter reading task did not reveal any significant difference. A whole-brain fMRI analysis revealed an enhanced task-specific activation of the hippocampal region bilaterally only in HD patients after the HD session. What is known regarding the role of the hippocampus in working memory? Although initial studies suggested that the hippocampus does not support working but only long-term memory,[14–16] more recent work showed that the hippocampi also subserve working memory.[17–19] The observed increase in neural activity in the hippocampal region associated with reduced reaction times may suggest that HD reduced hippocampal dysfunction in HD patients. This notion is corroborated by the behavioral data. HD patients had a tendency toward reduced reaction times and error rates after dialysis.

Further support for an influence of dialysis on memory functions is provided by our neuropsychological data. Memory is known to be related to a network including the temporal lobes.[20,21] In the figural memory test, healthy Con subjects improved their test results from t1 to t2, while HD patients did not show such a practice benefit. The results of the verbal learning and memory task differ from the figural memory task. Verbal learning is impaired in HD independent of the dialysis cycle. Results suggest that there is impairment in the neural memory network, at least at t1. Impaired verbal memory tests have been reported in dialysis patients previously.[22,23] The results of our neuropsychological verbal memory task do not reveal practice effects in patients or in healthy Cons.

In addition to the verbal memory impairment, HD patients revealed significant impairments of motor speed, task switching, verbal comprehension, word fluency, spatial visualization, spatial perception, and reasoning independent of the analyzed time point in comparison with the Con group. In addition, there was evidence for depression in HD patients. We did not find impairment in the verbal or spatial working memory tasks in the HD patients during neuropsychological testing at t1 or t2. Some of these impairments we found have already been reported in dialysis patients previously. Southeaver *et al.*[24] showed that uremic patients were impaired in visual alertness, flexible thinking, and speed of manipulation. Fazekas *et al.*[25] and Dahbour *et al.*[26] showed that chronic HD patients were impaired in different dementia scales (namley the Mattis Dementia Scale, Mini Mental State Examination). This, however, could not be replicated studying our sample of HD patients. Pliskin *et al.*[2,27] reported an increased incidence of depression in end-stage renal disease patients. Griva *et al.*[23] suggested that kidney transplant patients performed better than dialysis patients in verbal learning and attention. Lee *et al.*[28] showed that dialysis patients with higher hematocrit levels performed better in working memory and attention than patients with lower hematocrit levels. Murray *et al.*[3,23] found cognitive impairment in verbal and figural memory, attention, and depression.

Taken together, our own data and previous data suggest that HD patients suffer from significant cognitive impairment in multiple domains. However, at least for some of these cognitive domains, a dependency of time elapsed since dialysis has been suggested.

What are limitations of our study? Given the limited sample size we might have missed associations that would only be detectable in larger sample sizes. Owing to the small sample size and given that patients suffering from diabetes or neurological disorders were excluded, we cannot make any inferences beyond the sample studied here. To minimize the effects of daytime, we decided to perform all tests in precisely the same order at the same time on two consecutive days. Accordingly, we might have missed changes appearing at earlier time points, that is, within the first hours after dialysis. To maximize the potential effects of the metabolic disequilibrium, we studied the dialysis patients always at the end of a long weekend interval. We did not address cognitive changes in the course of shorter mid-week dialysis intervals. Furthermore, to maximize the potential effects of fluid shifts we selected dialysis patients with relatively large volume shifts in between dialysis sessions. Finally, given the published evidence that HD patients present with changes in verbal memory, attention, and working memory,[22,28,29,30] we studied a verbal working memory and a control letter reading task in the fMRI experiment. We would expect that activation changes in different brain regions might become detectable when studying different cognitive fMRI tasks.

Keeping all these limitations of this first fMRI study in dialysis patients in mind, the results of our study open an avenue for future research in this field. Studying an additional subgroup of HD patients with remaining urine production and absent ultrafiltration would address the effects of removal of uremic toxins versus removal of fluid on neuronal functions. The influence of dialysis efficacy could be studied in groups of HD patients with high versus low Kt/v and/or with short daytime versus long nocturnal dialysis. Finally, peritoneal dialysis patients might show completely different neuronal functional activation changes given the smaller amplitudes of fluid and metabolic changes in this group of dialysis patients.

In summary, in addition to showing cognitive impairment in dialysis patients in memory, attention, speech, and spatial abilities, we are the first to report specific changes of neural activation of memory-relevant areas in dialysis patients. Our findings of activation changes dependent on the dialysis cycle further indicate that hydration and/or metabolic fluctuations of dialysis patients in addition to the end-stage renal disease seem to have a significant effect on the level of neuropsychological functioning.

MATERIALS AND METHODS

Study design

The goal of this study was to identify differences in brain functions between chronic HD patients and healthy Cons by detailed neuropsychological testing and fMRI. For the fMRI part of the study, a verbal working memory task was chosen based on previous reports of impaired verbal and working memory in dialysis patients.[8,28] The fMRI data were analyzed as whole-brain analysis without any given assumption or pre-specified regions of interest. In all HD patients, assessment was started at the end of their long dialysis interval, that is, on a Monday morning (t1), with all patients having had their last HD treatment on the previous Friday. HD patients started their assessment at 0800 hours. The tests were always performed in the same order, starting with a blood draw and body weight assessment, followed by MRI measurements and then neuropsychological tests. The series of tests was finished by noon. In the afternoon, the patients underwent HD in their individual dialysis units. HD patients then spent the following night (i.e., from Monday to Tuesday) in an inpatient unit at the Research Center Jülich, Germany. The study measurements continued the following day at 0800 hours (t2). To eliminate potential daytime influences, all tests were performed in the same order as on the previous day. Healthy Con subjects were analyzed on two consecutive days (t1, t2) between 0800 hours and noon in the same test order as the HD patients.

Study participants

Stable, chronic HD patients ($n = 12$) were recruited in collaboration with regional dialysis centers (listed in the acknowledgments). Patient inclusion criteria were as follows: receiving chronic HD therapy for >6 months, hemoglobin >10 mg/dl for >3 months, stable blood pressure during the last HD session, significant fluid removal during the dialysis treatment (typically >2 l after a long weekend interval), and right handedness. Exclusion criteria included diabetes mellitus, presence of a known cerebral disease (i.e., previous brain trauma, cerebral ischemia, brain tumors, and hydrocephalus), presence of a known psychiatric disease, malignancies, chronic infectious diseases (i.e., chronic hepatitis B or C infection or human immunodeficiency virus infection), left handedness, anemia (hemoglobin <10 mg/dl over the past 3 months), hemodynamic instability during the past HD sessions ($>30\%$ decrease in systolic blood pressure during HD treatment), uncontrolled arterial hypertension ($>180/100$ mm Hg), metal-containing devices and implants (i.e., pacemakers, mechanical heart valves, implanted hearing aids, and artificial joint replacements), tattoos, and claustrophobia. Age- and sex-matched healthy Con subjects ($n = 12$) were recruited within the staff of the Research Center Jülich with subjects being naïve to the purpose of the study. HD patients and Cons received a financial reimbursement for their participation in the study. Informed consent was obtained from all participants. The study was approved by the local ethics committee (RWTH Aachen University, EK 064/06) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Blood chemistry

Blood parameters were analyzed in both groups at t1 and t2. Analyzed parameters included a blood count (including hemoglobin, hematrocrit, leukocytes, and platelets) as well as electrolytes (sodium and potassium). All measurements were performed in the same laboratory using routine clinical procedures.

Magnetic resonance imaging

MRI was carried out using echo planar imaging (EPI) with whole-brain coverage, excluding the cerebellum. fMRI was performed on a 1.5T Avanto system (SIEMENS, Erlangen, Germany), using the standard head coil for radio frequency transmission and signal reception. Sequences with the following parameters were used: repetition time $= 3000$ ms, echo time $= 60$ ms, field-of-view $= 200 \times 200$ mm^2, $\alpha = 90^\circ$, matrix size $= 64 \times 64$, voxel size $= 3.1 \times 3.1 \times 4.0$ mm^3. Using a mid-sagittal scout image, 30 axial slices, slice thickness $= 4$ mm (0.4 mm inter-slice gap), were positioned to cover the whole brain. The scanning procedure was performed continuously over each of the two experimental runs. In total, 226 scans per run were acquired. In addition, anatomical whole-brain images were obtained by using a T1-weighted, three-dimensional gradient-echo pulse sequence (magnetization-prepared, rapid-acquisition gradient-echo) with the following parameters: repetition time $= 2200$ ms, echo time $= 3.93$ ms, 15° flip angle, field-of-view $= 256 \times 256$ mm^2, matrix size $= 256 \times 256$, 160 sagittal slices with 1 mm thickness.

fMRI study – stimuli, tasks, and experimental design

Stimuli. A series of three different letters was shown in green in the middle of a white screen, using Arial font. Twelve letters (C, D, E, F, H, K, L, N, P, S, T, and V) were used to create the series by random rearrangement.[31] Each letter sustained a visual angle of $1.2^\circ \times 1.6^\circ$. Each of the three letters was presented for 500 ms.

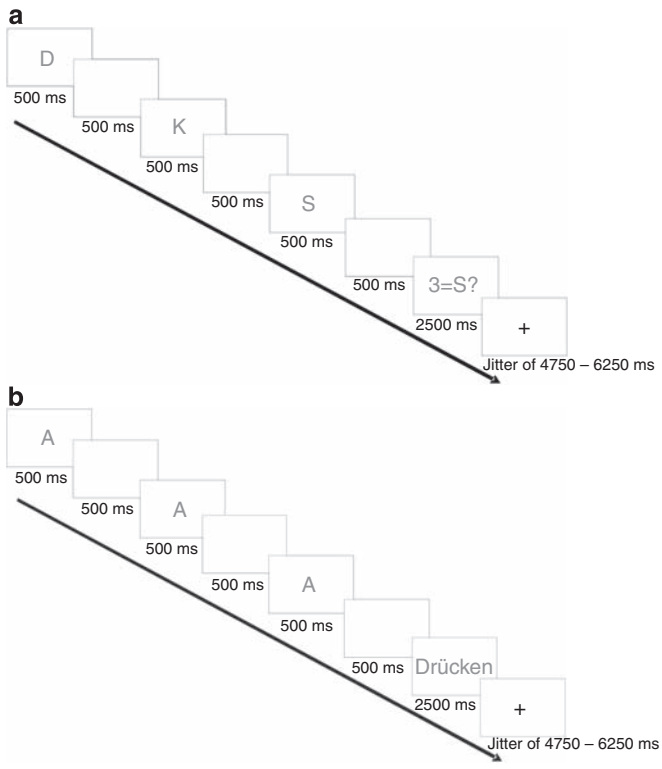

Figure 4 | Study design of the functional magnetic resonance imaging (fMRI) tasks. (**a**) Verbal working memory task: as an example of a task trial, after the presentation of a series of three different letters (D, K, S) the subject is asked whether the third letter of this series was an 'S'. (**b**) Control letter reading task: after the presentation of a series of three letters 'A' the subject is asked to press a button with the right index finger.

After each letter, a blank screen was presented for 500 ms before the next letter was presented (Figure 4a).

Working memory task. A task similar to the classical Sternberg paradigm was used. Subjects were asked to remember the letters and the positions of the letters presented in the series. Immediately after the presentation of the last letter, a question, for example: '3 = S?', was presented in green in the middle of the white screen for 2500 ms. The question thus asked subjects whether, for example, the third letter that was presented in the series had been an S. The question sustained a visual angle of $3.1^\circ \times 1.6^\circ$. Subjects were instructed to answer 'Yes' by pressing a button with their index finger and 'No' by pressing a button with their middle finger of the left (or right) hand. After the first of two experimental runs, the subjects were asked to change the hand for response. The starting hand was randomized over subjects. Total measurement time per run was 11.3 min. After the presentation of the question, a fixation cross was presented for 4750, 5250, 5750, or 6250 ms (randomly varied between these to increase variance in the imaging time-series).

Each run started with the introduction, 'Welcome to the letter test. Duration approximately 10 min', for 3000 ms. After that, the instructions, 'Please use your right (left) hand only. Yes → index finger, No → middle finger.' were presented for 10,000 ms. Next, the word 'START' was shown in black in the middle of the white screen for 500 ms. During one run of the trials, the subjects were questioned 12 times for each of the serial position of the letter presented. In 50% of the responses, the position of the letters presented in the question was correct. If the position in question was incorrect, the letter had indeed been shown in that particular series of letters, however, in another position. In each of the two runs, a control task was implemented.

Control task. As fMRI control task we used a letter reading task. During this task, a series of three A's was presented for 500 ms each in the middle of the screen in red on a white background

Table 3 | Description of neuropsychological tests

Function	Test	Reference	Description
Working memory			
Verbal	Subtest of Wechsler intelligence scale (German version) (WIE)	Aster *et al.*[42]	Digit span Rehearsal of spoken digit rows
Spatial	Block tapping test (BTT)	Schelling[43]	Reproduction of a sequence of tappings on a set of nine wooden blocks
Verbal memory			
Learning, recall, recognition	Rey auditory verbal learning test (German version) (VLMT)	Helmstaedter *et al.*[44]	Word list learning, recall, and recognition
Figural memory			
	Diagnosticum of cerebral damage (DCS)	Weidlich and Lamberti[45]	Reproduction of previously shown figures with wooden sticks
Attention			
Visuomotor speed	Trail making test (TMT-A) of the Halstead–Reitan neuropsychological test battery: therapy and clinical interpretation	Reitan and Wolfson[46]	Connecting numbers in an ascending sequence
Task switching	Trail making test (TMT-B) of the Halstead–Reitan neuropsychological test battery: therapy and clinical interpretation	Reitan and Wolfson[46]	Connecting numbers and letters alternating in an ascending sequence
Interference	Farbe-Wort-Interferenztest (FWIT), a German version of the Stroop test	Bäumler[47]	Naming of the color of a written color word
Speech			
Verbal comprehension	Leistungsprüfsystem (LPS) Subtest of a German intelligence scale	Sturm *et al.*[48] and Horn[49]	Identification of spelling mistakes in words with ascending level of familiarity
Word fluency	Leistungsprüfsystem (LPS) Subtest of a German intelligence scale	Sturm *et al.*[48] and Horn[49]	Producing of words with a given initial
Naming	Aachener Aphasie test	Huber *et al.*[50]	Naming of drawn objects or scenes
Perception			
Spatial visualization	Leistungsprüfsystem (LPS) Subtest of a German intelligence scale	Sturm *et al.*[48] and Horn[49]	Identification of a mirror-inverted letter of number between others
Perceptual speed	Leistungsprüfsystem (LPS) Subtest of a German intelligence scale	Sturm *et al.*[48] and Horn[49]	Identification of embedded figures within geometrical figures
Reasoning			
	Leistungsprüfsystem (LPS) Subtest of a German intelligence scale	Sturm *et al.*[48] and Horn[49]	Identification of rules in a row of geometrical figures
Dementia	Mini-mental-status-test (MMST) (German version)	Kessler *et al.*[51]	Test battery
Depression	Beck Depression Inventory (BDI) (German version)	Hautzinger *et al.*[52]	Self-rating questionnaire
Personality	Freiburger Personality Inventory–Revised (FPI-R)	Fahrenberg *et al.*[53]	Self-rating questionnaire

(Figure 4b). After each letter, a blank screen was presented for 500 ms. Thereafter the German word for 'press' (drücken) appeared in red on a white screen for 2500 ms. Subjects were instructed to press the button with the index finger after the word 'press' was shown. Finally, a so called low-level baseline was implemented in the series by presenting a fixation cross for 5500 ms. At the end of each run, 'END' and 'Thank you for your efforts.' (in German) appeared on the screen for 10,000 ms.

Image processing

Images were analyzed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm) as follows. The EPI images were corrected for head movement between scans by an affine registration.[32] For realignment we used a two-pass procedure, by which images were initially realigned to the first image of the time-series and subsequently re-realigned to the mean of all images after the first step. After completing the realignment, the mean EPI image for each subject was computed and spatially normalized to the MNI single-subject template[33–35] using the 'unified segmentation' function in SPM5. This algorithm is based on a probabilistic framework that enables image registration, tissue classification, and bias correction to be combined within the same generative model. The resulting parameters of a discrete cosine transform, which defines the deformation field necessary to move the subject's data into the space of the MNI tissue probability maps,[36] were then combined with the deformation field transforming between the latter and the MNI single-subject template. The ensuing deformation was subsequently applied to the individual EPI volumes as well as to the T1 scan, which was coregistered to the mean of the realigned EPIs beforehand. All images were hereby transformed into standard stereotaxic space and resampled at $2 \times 2 \times 2\,mm^3$ voxel size. The normalized images were spatially smoothed using an 8 mm full width at half maximum Gaussian kernel to meet the statistical requirements of the general linear model and to compensate for residual macroanatomical variations across subjects.

The data were analyzed in a whole-brain analysis (excluding the cerebellum) using a general linear model as implemented in SPM5.[37] Each experimental condition was modeled using a boxcar reference vector convolved with a canonical hemodynamic response function and its first-order temporal derivative by using miniblocks, starting at the presentation of the first letter and lasting till the response or, if no response occurred, for 5500 ms. Incorrect trials, or a trial in which no answer occurred, were modulated on a separate vector. Low-frequency signal drifts were filtered using a cutoff period of 128 s. Parameter estimates were subsequently calculated for each voxel using weighted least squares to provide maximum likelihood estimators based on the temporal autocorrelation of the data[37] to get identical and independently distributed error terms. No global scaling was applied. For each subject, simple main effects for each experimental condition were analyzed in comparison with the low-level baseline. These first-level individual contrasts were then fed into a second-level group analysis using analysis of variance (factor: time point: t1, t2, group: HD, Con, blocking factor subject) using a random-effects model.[38] In the modeling of variance components, we allowed for violations of sphericity by modeling non-independence across parameter estimates from the same subject and allowing unequal variances both between conditions and subjects using the standard implementation in SPM5.

Contrasts. Areas of activation were identified as significant only if they passed a threshold of $P_c < 0.05$, corrected for multiple comparisons at the cluster level, with an underlying voxel level of $P \leqslant 0.001$ (uncorrected). Significant results are only reported for the cerebrum. The main effects of time (t1, t2) and group (HD, Con) were calculated by a conjunction analysis of t1 and t2 or respectively of HD patients or Cons. In addition, the interactions between group and time were calculated on the second level.

Functional activations were anatomically localized using the version 1.5 of the SPM anatomy toolbox[39,40] (http://www.fz-juelich.de/ime/spm_anatomy_toolbox) using a maximum probability map. This map[41] denotes the most likely anatomical area at each voxel of the MNI single-subject template based on probabilistic cytoarchitectonic maps derived from the analysis of cortical areas in a sample of 10 human post-mortem brains, which were subsequently normalized to the MNI reference space.

Neuropsychological assessment

HD patients and Cons were analyzed extensively at t1 and t2 with standardized neuropsychological tests covering 10 different cognitive domains including working memory, verbal learning and memory, figural memory, attention, speech, perception, reasoning, dementia (screening), depression, and personality. Detailed information regarding each test is provided in Table 3. Parallel test versions of the Verbaler Lern-und Merkfähigkeitstest (VLMT), the Diagnosticum of cerebral damage, and the Leistungsprüfsystem were used at t2. The duration of the neuropsychological investigation was approximately 2 h. Testing was performed immediately after the fMRI scanning procedure in a test room of the Institute of Neurosciences and Medicine at the Research Center Jülich. The raw values of the neuropsychological test results were transformed into standard *t*-values (mean of 50 and s.d. of 10), if possible.

Statistical analysis

Statistical Package of the Social Sciences Version 17, SPSS GmbH Software, an IBM Company, München, Germany was used to perform parametric group statistics on clinical data, descriptive data, behavioral data (reaction times, error rates) of the fMRI experiment, and neuropsychological data. A general linear model (analysis of variance) with the factors TIME (t1 and t2) as within-subject factor and GROUP (HD and Con) as between-subject factor was assessed by calculating the respective F-tests (F). *Post hoc T*-tests were carried out to Con for the direction of significant differences (e.g., group differences, differences of time, or interactions) within the analysis of variance. If data were only available for one time point *T*-tests (T) were calculated.

DISCLOSURE

All the authors declared no competing interests.

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